Clinical Reviews in Allergy & Immunology (2022) 63:342–356
https://doi.org/10.1007/s12016-022-08935-z

Serum Biomarkers for Autoimmune Hepatitis Type 1: the Case for CD48 and a Review of the Literature

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Accepted: 10 March 2022 / Published online: 3 June 2022

Abstract

In autoimmune hepatitis (AIH), the persisting inflammation contributes to fibrosis progression, for which conventional biochemical markers manifest relatively unsatisfactory prediction. Herein, we assessed the value of serum CD48 (sCD48) as an indicator for inflammation and fibrosis in AIH type 1. The levels of sCD48 were detected first in an exploratory cohort using ELISA. In this cohort, compared with healthy controls (4.90 ng/mL, $P < 0.0001$), primary biliary cholangitis (7.32 ng/mL, $P < 0.0001$), and non-alcoholic fatty liver disease (7.76 ng/mL, $P < 0.0001$), sCD48 levels were elevated in AIH (12.81 ng/mL) and correlated with histological inflammation and fibrosis. Further using multivariate logistic regression analysis, sCD48 was identified as an independent predictor for both significant inflammation (G3-4) and advanced fibrosis (S3-4). Two predictive scores, based on sCD48, were constructed for diagnosing significant inflammation and advanced fibrosis (sCD48-AIH-SI and sCD48-AIH-AF, respectively). Using these data as a premise, predictive abilities were subsequently evaluated and verified in a validation cohort. In the exploratory cohort, the area under the receiver operating characteristic curve of sCD48 and sCD48-AIH-SI, for significant inflammation, were 0.748 and 0.813, respectively. Besides, during treatment follow-up, sCD48 levels gradually decreased from immunosuppression initiation to re-evaluation biopsy, in parallel with aspartate transaminase, total sera IgG, and fibrosis-4 score. For AIH patients in a re-evaluation biopsy cohort, sCD48 could predict significant fibrosis (S2-4). Further using immunohistochemistry, hepatic CD48 expression was elevated in AIH patients and decreased after treatment. In conclusion, sCD48 and sCD48-based predictive scores predict histological inflammation and fibrosis in AIH-1. Detecting sCD48 might help in the clinical management of AIH.

Keywords Autoimmune hepatitis · Serum CD48 · Indicator · Hepatic inflammation · Liver fibrosis

Abbreviations

AIH	Autoimmune hepatitis
ALB	Albumin
ALT	Alanine transaminase
AST	Aspartate transaminase
ALP	Alkaline phosphatase
ANA	Antinuclear antibody
APRI	Aminotransferase-to-platelet ratio index
ASMA	Smooth muscle antibody
Anti-SLA/LP	Soluble liver antigen/liver pancreas antigen antibodies
AUC	Area under receiver operating characteristic curve
AZA	Azathioprine
FIB-4	Fibrosis-4 score
GGT	Gamma-glutamyl transpeptidase
INR	International Normalized Ratio
IgG	Immunoglobulin G
LSM	Liver stiffness measurement
PLT	Platelet

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PT	Prothrombin time
ROC	Receiver operating characteristic curve
sCD48	Serum CD48
sCD48-AIH-SI	SCD48-based predictive model for significant inflammation in AIH
sCD48-AIH-AF	SCD48-based predictive model for advanced fibrosis in AIH
TBIL	Total bilirubin
TE	Transient elastography

Introduction

In the clinical management of AIH, the selection and adjustments in therapy should be individualized based upon drug-related side effects and disease activity [1, 2]. Liver biopsy remains the gold standard for assessing hepatic inflammation and fibrosis, but due to invasiveness, expense, possible sampling error, and complications, its clinical applicability is limited, especially for assessment during long-term follow-up [3]. Although serum aminotransferase and IgG are routinely detected for monitoring diseases activity, they do not reliably reflect histological inflammation [4, 5]. Transient elastography (TE) demonstrates the reasonable discriminative ability for fibrosis but is influenced by hepatic inflammation, obesity, and operator skill [6, 7]. Recently, noninvasive markers for histological findings have been explored for AIH, including serum Wisteria floribunda agglutinin positive Mac-2-binding protein (WFA^+-M2BP), serum vitamin D, and gut microbiome analysis [8–12]. Clearly, there is an unmet need for newer biomarkers. CD48, a member of the signaling lymphocyte activation molecule family, is constitutively expressed on most hematopoietic cells, and under inflammatory conditions, CD48 expression is increased on stimulated subsets of cells [13]. CD48 participates in T-cell activation signaling, immune synapse organization, and target lysis by effector lymphocytes, which have been reported in various diseases including chronic hepatitis B and hepatocellular carcinoma [13–15]. Apart from the membrane-bound form, a soluble form of CD48 has been found [16]. The value of soluble CD48 in serum or plasma as biomarkers of disease activity has been studied in immune-mediated arthritis, asthma, and Sjögren's syndrome [17–19]. The objective herein was to evaluate the utility of sCD48 for predicting histological inflammation and fibrosis in AIH-1.

Materials and Methods

Our study enrolled a total of 293 AIH-1 patients diagnosed at the Department of Gastroenterology and Hepatology of Shanghai Renji Hospital between December 2013 and May 2021 (Supporting Fig. S1A). Profiles of the cohorts studied are found in Supporting Table S1. Of the total AIH patients, 221 participants were newly diagnosed and the inclusion criteria were (i) a diagnosis of probable or definite AIH based on the simplified scoring system for AIH [20]; (ii) liver histologic analysis and serum samples collected at the time of liver biopsy; (iii) the absence of concurrent liver diseases; (iv) absence of anti-liver-kidney-microsome antibodies and/or anti-liver cytosol antibody type 1 antibodies (AIH type 2); (v) immunosuppression naïve before liver biopsy and serum collection. After diagnosis, all patients were treated with prednisolone to induce remission and then a low dose of prednisolone or in combination with AZA/mycophenolate mofetil as maintenance therapy. A re-evaluation of liver biopsy was performed in patients after around 3-year treatment. During the treatment courses, 66 patients with treatment-naïve serum samples collected at diagnostic biopsy have been followed up (Supporting Fig. S1B).

Apart from the 221 newly diagnosed participants, 72 AIH patients at re-evaluation liver biopsy were included, whose serums at baseline could not be attained. The inclusion criteria for these patients were identical to that of newly diagnosed ones except that they had received immunosuppressive therapy for around 3 years before the re-evaluation biopsy. Otherwise, 50 patients with primary biliary cholangitis (PBC) and 29 patients with non-alcoholic fatty liver disease (NAFLD) were recruited as disease controls, both with standard diagnosis meeting corresponding criteria [21, 22]. Healthy controls (HC, $n = 39$) were also included, all with normal liver function tests and no positivity of hepatitis B/C virus antigen. Serum samples were available in all disease and healthy controls. Then, with all the patients and HC recruited, three study cohorts were established (Supporting Fig. S1A) that is the exploration cohort ($n = 268$, including 150 naïve AIH patients, 50 PBC patients, 29 NAFLD patients, and 39 HC), validation cohort ($n = 71$, naïve AIH patients) and re-evaluation biopsy cohort ($n = 114$, AIH patients at re-evaluation biopsy). All participants enrolled provided written informed consent, and the study protocol was approved by the Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (No.2013–030).

Serum samples were stored at –80 °C until detection. Laboratory tests were conducted using accredited laboratory procedures. TE was performed prior to liver biopsy on the same day, as described before [23]. Two fibrosis scores, aminotransferase-to-platelet ratio index (APRI) and fibrosis-4 score (FIB-4), were calculated based on reported formulas respectively [24, 25].

Liver Histology Examination

Two experienced histopathologists, who were blinded to patients' clinical characteristics, independently evaluated

the histological inflammation and fibrosis in reference to the Scheuer system [26].

Measurement of the Serum Level of Soluble CD48

The level of sCD48 was detected via a human soluble CD48 enzyme-linked immunosorbent assay kit (KIT10797, Sino Biological Inc., Beijing, China), according to the operating instructions.

Immunohistochemical Staining of Liver Biopsies

Paraffin-embedded liver biopsy tissues were obtained from randomly selected 37 AIH patients, 11 PBC patients, 12 NAFLD patients, and 8 HC in the exploration cohort. Liver biopsy samples were also obtained from 11 of the 37 AIH patients followed up at re-evaluation biopsy. The antibody for CD48 (ab134049, Abcam, Cambridge, UK) was used as the primary antibody. Immunohistochemistry was performed as described before [27]. Liver sections were examined via a light microscope (Olympus, Japan). Five fields were randomly selected per case and the expression degree of CD48 was scored semi-quantitatively from 0 to 4 per high-power field (40 × 10 magnification): 0 if positive area < 5%, 1 if ≥ 5–< 25%, 2 if ≥ 25–< 50%, 3 if ≥ 50–< 75%, and 4 if ≥ 75%. The final result was the mean of scores five fields got.

Statistical Analysis

Continuous variables and categorical data were respectively expressed as medians with range and frequencies with percentages, unless otherwise indicated. The comparison between continuous variables was analyzed by the Mann–Whitney *U* test. Categorical variables were assessed by chi-squared test or Fisher' s exact test, as appropriate. Correlations between two variables were performed by Spearman rank correlation. The univariate analysis was performed via chi-square/Fisher's exact test and Mann–Whitney *U* test. Variables with $P < 0.05$ in the univariate analysis were then included in the multivariate analysis, except for those not available in all patients or not clinically significant, as noted. Multivariate analysis was conducted using the binary forward stepwise logistic regression to identify the independent predictors and construct predictive scores. The analysis of receiver operator characteristic curves (ROC) was obtained by Delong's method. The cutoff values were determined by the Youden index. All analyses were performed via IBM SPSS Statistics 25.0 (IBM, Chicago, IL) and MedCalc18.2.1. $P < 0.05$ was considered statistically significant and all *p* values were two-tailed.

Results

Elevated sCD48 Levels Correlate with Histological Severity in Naïve AIH Patients

Detailed data on immunosuppressive treatment-naïve AIH patients in both the exploration cohort and validation cohort were listed in Table 1. In the exploration cohort, the level of sCD48 was assessed in 150 patients with AIH, 29 with NAFLD, 50 with PBC, and 39 HC (Fig. 1A). When compared with HC (4.90, IQR 4.12–6.67 ng/mL), the median level of sCD48 was significantly higher in AIH patients (12.81, IQR 9.36–17.21 ng/mL, $P < 0.0001$), NAFLD patients (7.76, IQR 5.82–11.99 ng/mL, $P = 0.0002$), and PBC patients (7.32, IQR 5.13–11.52 ng/mL, $P = 0.0002$). However, relative to NAFLD and PBC patients, sCD48 levels were elevated in AIH (both *P* values < 0.0001). sCD48 demonstrated a significantly positive association with both inflammation grades and fibrosis stages ($r = 0.411$, $P < 0.0001$ and $r = 0.324$, $P < 0.0001$ respectively; Fig. 1B). The median sCD48 level increased with the severity of inflammation as 9.49 ng/mL (G1), 9.95 ng/mL (G2), 15.64 ng/mL (G3), and 18.51 ng/mL (G4). There was a significant difference in sCD48 levels between AIH patients with G2 vs G3 ($P < 0.0001$, Fig. 1B). However, due to the relatively few patients in subgroups of G1 and G4 (both with 4 cases), no statistical difference of sCD48 levels was observed between AIH patients with G1 vs G2 or G3 vs G4. Likewise, sCD48 levels were elevated and correlated with fibrosis stages, 8.84 ng/mL (S1), 11.47 ng/mL (S2), 15.45 ng/mL (S3), and 16.73 ng/mL (S4). The difference between patients with S1 vs S2 and S2 vs S3 was statistically significant (both $P < 0.05$, Fig. 1B). sCD48 levels positively correlated with ALT, AST, and IgG (Supporting Table S2).

Predictor Analysis for Significant Histological Inflammation

Then, the predictive ability of sCD48 for inflammation grades was investigated in naïve AIH patients. As mentioned above, AIH patients with G1 and G4 were both only 4 cases in the exploration cohort, and thus, we dichotomized these AIH patients into groups with mild to middle inflammation (G1-2, $n = 54$) and significant inflammation (G3-4, $n = 96$). AIH patients in the significant inflammation group had a markedly higher level of sCD48 (15.68, IQR 11.46–19.10 ng/mL) than the other one (9.88, IQR 8.07–11.77 ng/mL, $P < 0.0001$, Fig. 1C). According to the univariate analysis, other indices statistically different between the two groups included AST,

Table 1 Profiles of naïve AIH patients in the exploration and validation cohort

Variable	Exploration cohort ($n=150$)	Validation cohort ($n=71$)	*P* value
Age (years)	51 (16–73)	51 (18–71)	0.673
Gender, female (%)	128 (85.3%)	62 (87.3%)	0.691
Serum CD48 (ng/mL)	12.81 (4.58–35.92)	12.75 (2.19–41.10)	0.771
Liver function test			
ALT (U/L)	72 (9–987)	81 (8–826)	0.798
AST (U/L)	63.5 (10–657)	75 (15–723)	0.191
ALP (U/L)*	99 (46–309)	99 (24–190)	0.539
GGT (U/L)*	59.1 (6–403)	64 (12–733)	0.656
TBIL (μmol/L)	14.9 (2.9–123.1)	13.6 (4.8–78.2)	0.226
ALB (g/L)	40.9 (28.0–50.8)	41.5 (29.3–49.3)	0.701
Immunoglobulin			
IgG (g/L)	17.4 (8.4–41.6)	17.3 (7.7–45.4)	0.909
IgM (g/L)	1.22 (0.31–4.11)	1.25 (0.27–4.54)	0.789
Blood routine test			
WBC (10^9/L)	4.92 (1.90–10.95)	4.77 (1.95–10.63)	0.305
Hb (g/L)	128 (67–172)	127 (85–159)	0.663
PLT (10^9/L)	184.5 (41–439)	175 (62–463)	0.649
Autoantibody			
ANA +, *n* (%)	116 (77.3%)	54 (76.1%)	0.865
ASMA +, *n* (%)*	7 (4.8%)	8 (11.4%)	0.090
Anti-SLA/LP +, *n* (%)*	2 (1.4%)	3 (4.2%)	0.334
Coagulation test			
PT (s)	12.0 (8.1–16.8)	11.8 (9.6–16.5)	0.611
INR	1.03 (0.72–1.30)	1.02 (0.82–1.47)	0.845
Fibrosis indicators			
APRI	0.97 (0.11–9.58)	1.25 (0.16–9.82)	0.212
FIB-4	2.26 (0.41–25.70)	2.49 (0.34–15.69)	0.069
LSM (kPa)*	11.8 (3.4–35.8)	9.6 (3.7–27.4)	0.105
Histological findings			
Inflammation grade, G1/2/3/4, *n* (%)	4/50/92/4 (2.7/33.3/61.3/2.7)	0/34/36/1 (0.0/47.9/50.7/1.4)	0.127
Fibrosis stage, S1/2/3/4, *n* (%)	10/68/54/18 (6.7/45.3/36.0/12.0)	4/40/20/7 (5.6/56.3/28.2/9.9)	0.532
Clinical characteristics			
Concurrent diseases, *n* (%)**	24 (16.0%)	17 (23.9%)	0.156

*ALP and GGT were both available in 145 and 68 patients for the exploration cohort and the validation cohort respectively; ASMA and SLA/LP were both available in 145 and 70 patients for the exploration cohort and the validation cohort respectively; LSM by TE was available in 51 and 46 patients for the exploration cohort and the validation cohort respectively; **Concurrent diseases include autoimmune diseases (autoimmune thyroiditis, Sjogren syndrome, type 1 diabetes, rheumatoid arthritis, and psoriasis) and allergic disease (asthma, allergic rhinitis, and eczema). Continuous variables and categorical data were expressed as medians with range and frequencies with percentages, respectively

alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin, albumin (ALB), IgG, and PLT, all of which were then taken into a multivariate analysis except for ALP and GGT, as they were not available in all patients. Finally, sCD48, IgG, and PLT were demonstrated to be independent predictors for significant inflammation (Supporting Table S3), with which a sCD48-based prediction model for significant inflammation was constructed as follows: $\text{sCD48-AIH-SI} = 1/(1 + e^{1.305-0.139\times\text{sCD48}-0.122\times\text{IgG}+0.01\times\text{PLT}})$ (Hosmer–Lemeshow test: $\chi^2 = 10.741$, $P = 0.217$).

Thence, we examined the discriminative value of sCD48 and sCD48-AIH-SI for significant inflammation in AIH patients in the exploration cohort (Fig. 1D; Table 2). The area under the receiver operating characteristic curve (AUC) of sCD48 was 0.748, which was statistically higher than that of ALT (0.584, $P = 0.0048$) and demonstrated an increasing but not significant trend relative to AST (0.679, $P = 0.1698$) and IgG (0.677, $P = 0.2009$). With respect to sCD48-AIH-SI, its AUC was 0.813, superior than ALT ($P = 0.0001$), AST ($P = 0.0126$), IgG ($P = 0.0008$) and also sCD48 ($P = 0.0406$). The predictive efficacy, confirmed

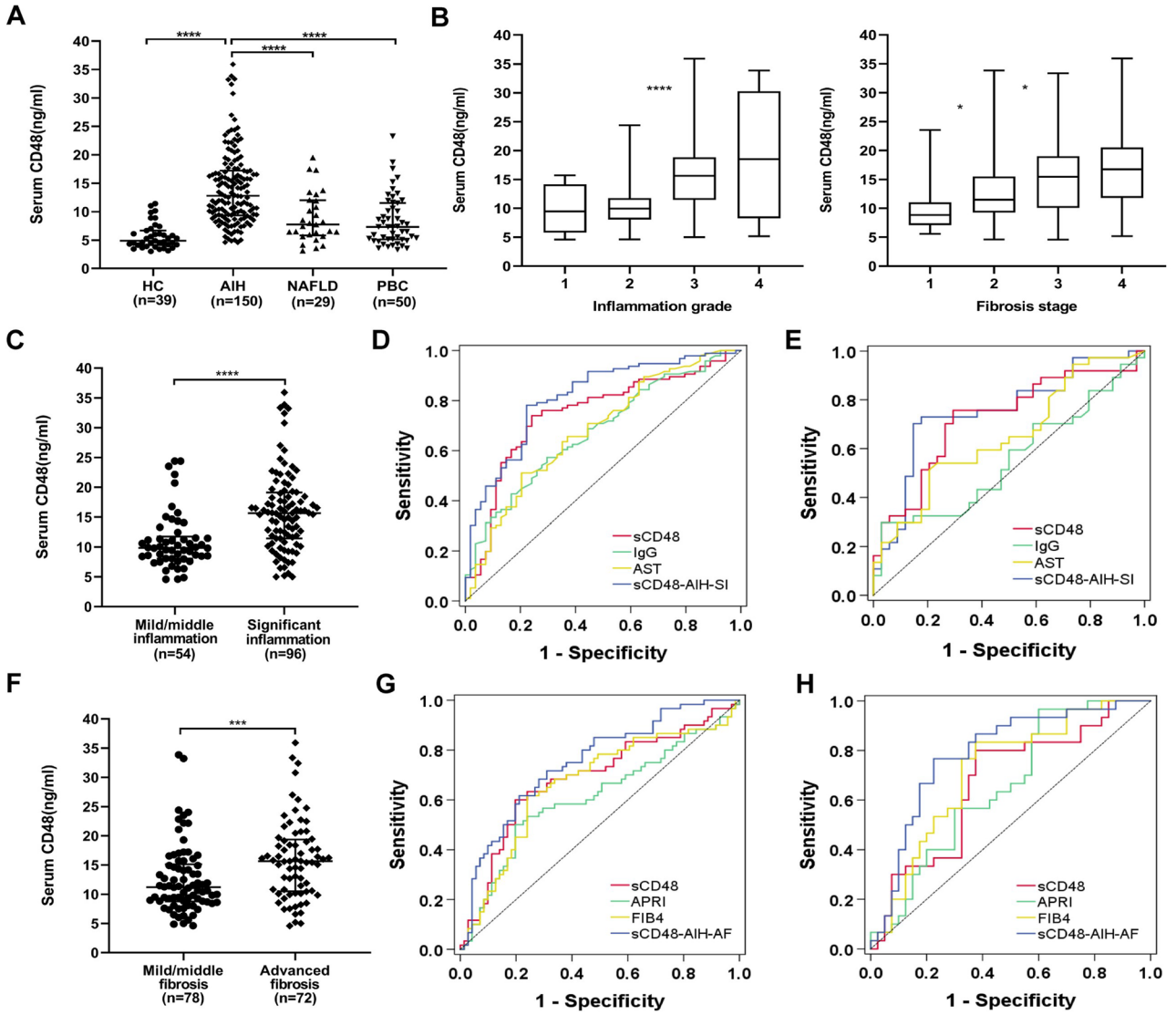


Fig. 1 sCD48 levels were elevated in naïve AIH patients and could predict significant inflammation and advanced fibrosis. **A** sCD48 levels in the exploration cohort, including immunosuppressive treatment-naive AIH patients ($n=150$), NAFLD patients ($n=29$), PBC patients ($n=50$), and healthy controls ($n=39$). **B** left, correlation between sCD48 levels and liver inflammation grades ($r=0.411$, $P<0.0001$), $^{****}P<0.0001$ for patients with G2 vs G3; right, correlation between sCD48 levels and liver fibrosis stages ($r=0.324$, $P<0.0001$), $^{*}P<0.05$ for patients with S1 vs S2 and S2 vs S3. **C** sCD48 levels in AIH patients with mild to middle inflammation (G1-2) and significant inflammation (G3-4). **D**, **E** ROC of sCD48, sCD48-AIH-SI, and clinical indices for detecting significant inflammation in naive AIH patients of the exploration cohort ($n=150$, **D**) and the validation cohort ($n=71$, **E**). **F** sCD48 levels in AIH patients with mild to middle fibrosis (S1-2) and advanced fibrosis (S3-4). **G**, **H** ROC of sCD48, sCD48-AIH-AF, APRI, and FIB-4 for detecting advanced fibrosis in naive AIH patients of the exploration cohort ($n=150$, **G**) and the validation cohort ($n=71$, **H**). Bars or boxes represented the median with interquartile ranges. $^{****}P<0.0001$, $^{***}P<0.001$

in the validation cohort, included an AUC of 0.724 for sCD48 and an AUC of 0.753 for sCD48-AIH-SI to identify significant inflammation (Fig. 1E; Table 2). Overall, these findings suggested that in naïve AIH patients, sCD48 and sCD48-AIH-SI might be powerful indicators for significant inflammation.

Predictor Analysis for Histological Advanced Fibrosis

We further explored the diagnostic potential of sCD48 for fibrosis in naïve AIH patients. In view of the small number of patients in subgroups with S1 ($n=10$) and S4 ($n=18$), we divided AIH patients in the exploration cohort into groups

Table 2 ROC analysis of significant inflammation in naïve AIH patients of the exploration cohort ($n=150$) and the validation cohort ($n=71$)

Parameters	Cutoff	AUC (95% CI)	Sen (%)	Spe (%)	PPV (%)	NPV (%)	+LR	−LR	*P* value
Exploration cohort									
sCD48 (ng/mL)	11.54	0.748 (0.665–0.831)	73.96	75.93	84.5	62.1	3.07	0.34	< 0.0001
IgG (g/L)	18.90	0.677 (0.590–0.764)	43.01	82.46	80.0	47.0	2.45	0.69	< 0.0001
ALT (U/L)	33	0.584 (0.487–0.681)	81.25	35.19	69.0	51.4	1.25	0.53	0.089
AST (U/L)	82	0.679 (0.590–0.769)	51.04	79.63	81.7	47.8	2.51	0.61	< 0.0001
sCD48-AIH-SI	0.61	0.813 (0.742–0.884)	78.12	77.78	86.2	66.7	3.52	0.28	< 0.0001
Validation cohort									
sCD48 (ng/mL)	12.37	0.724 (0.604–0.844)	75.68	70.59	73.7	72.7	2.57	0.34	0.001
IgG (g/L)	23.2	0.553 (0.417–0.688)	29.73	97.06	91.7	55.9	10.11	0.72	0.444
ALT (U/L)	127	0.583 (0.450–0.716)	35.14	82.35	68.4	53.8	1.99	0.79	0.229
AST (U/L)	103	0.649 (0.522–0.777)	51.35	79.41	73.1	60.0	2.49	0.61	0.030
sCD48-AIH-SI	0.75	0.753 (0.636–0.869)	70.27	85.29	83.9	72.5	4.78	0.35	< 0.0001

CI confidence interval, *Sen* sensitivity, *Spe* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *+LR* positive likelihood ratio, *−LR* negative likelihood ratio

with mild to middle fibrosis (S1-2, $n=78$) and advanced fibrosis (S3-4, $n=72$), and AIH patients with advanced fibrosis manifested higher sCD48 levels (Fig. 1F). Thence, multivariate analysis was conducted with sCD48 and other variables different between the two groups, including age, ALB, PLT, prothrombin time (PT), international normalized ratio (INR), APRI, and FIB4 (Supporting Table S4). Noteworthy, TE was not available in a large proportion of patients ($n=99$) and thus has not been included in the multivariate analysis. Finally, sCD48, PT, and age presented independent predictive value for advanced fibrosis (Supporting Table S4), which were then used to construct a sCD48-based predictive score for advanced fibrosis: $\text{sCD48-AIH-AF} = 1/(1+e^{8.157-0.083\times\text{sCD48}-0.406\times\text{PT}-0.038\times\text{age}})$ (Hosmer–Lemeshow test: $\chi^2=7.382$, $P=0.496$).

The discriminative ability of sCD48 and sCD48-AIH-AF for advanced fibrosis was firstly evaluated in AIH patients in the exploration cohort (Fig. 1G; Table 3). The AUC of sCD48 was 0.686, similar to those of APRI (0.611) and FIB-4 (0.667). While sCD48-AIH-AF showed an AUC of 0.750, significantly superior than APRI ($P=0.0026$) and FIB-4 ($P=0.0299$), but not sCD48 ($P=0.1014$). In the validation cohort (Fig. 1H; Table 3), the AUCs of sCD48 and sCD48-AIH-AF for predicting advanced fibrosis were 0.669 and 0.788 respectively. Moreover, for AIH patients with significant fibrosis (S2-4) and cirrhosis (S4), sCD48 also showed discriminative value (Supporting Table S5). Taken together, compared with FIB-4 and APRI, sCD48 and sCD48-AIH-AF demonstrated a comparable or even superior performance for identifying advanced fibrosis in AIH patients.

Longitudinal Change of sCD48 Levels During Treatment Follow-up

In the follow-up of newly diagnosed AIH patients, we observed that sCD48 levels decreased after 6-month immunosuppressive treatment ($n=35$, Fig. 2A). Additionally, in

Table 3 ROC analysis of advanced fibrosis in naïve AIH patients of the exploration cohort ($n=150$) and the validation cohort ($n=71$)

Parameters	Cutoff	AUC (95% CI)	Sen (%)	Spe (%)	PPV (%)	NPV (%)	+LR	−LR	*P* value
Exploration cohort									
sCD48 (ng/mL)	14.93	0.686 (0.592–0.780)	60.00	80.28	72.0	70.4	3.04	0.50	< 0.0001
APRI	1.46	0.611 (0.514–0.708)	50.00	80.28	68.2	65.5	2.54	0.62	0.030
FIB4	2.61	0.667 (0.571–0.763)	61.67	76.06	68.5	70.1	2.58	0.50	0.001
sCD48-AIH-AF	0.44	0.750 (0.666–0.833)	71.67	69.01	66.2	74.2	2.31	0.41	< 0.0001
Validation cohort									
sCD48 (ng/mL)	12.37	0.669 (0.540–0.798)	80.00	62.50	61.5	80.6	2.13	0.32	0.016
APRI	0.60	0.656 (0.529–0.783)	96.67	40.00	54.7	94.1	1.61	0.083	0.026
FIB4	2.12	0.718 (0.597–0.838)	83.33	62.50	62.5	83.3	2.22	0.27	0.002
sCD48-AIH-AF	0.41	0.788 (0.679–0.898)	76.67	77.50	71.9	81.6	3.41	0.30	< 0.0001

patients with paired diagnostic and re-evaluation biopsy, sCD48 levels were significantly lower at the later time point ($n=42$, Fig. 2B). We also observed Δ serum CD48 (Δ = the value of diagnostic biopsy–the value of re-evaluation biopsy) manifested a positive association with Δ inflammation grade ($r=0.322$, $P=0.0376$, Fig. 2C). The longitudinally dynamic change of sCD48 levels during treatment was similar to IgG and AST (Fig. 2D). However, sCD48 levels did not reflect biochemical remission after 6-month and 12-month treatment and at re-evaluation biopsy (data not presented). Additionally, different from AST and IgG, which normalized in most cases at 12 months after treatment and re-evaluation biopsy, sCD48 maintained a higher level in most cases (relative to HC in this study). Noteworthy, though Δ serum CD48 did not correlate with Δ fibrosis stage ($r=0.042$, $P=0.7924$, Fig. 2C), the dynamic change of sCD48 resembled that of FIB-4. In all, sCD48 reflected disease activity and possibly histological inflammation resolution during treatment follow-up.

sCD48 Levels Predict Significant Fibrosis in AIH Patients at Re-evaluation Biopsy

Additionally, we investigated sCD48 in a cohort comprising 114 AIH patients at re-evaluation biopsy. The median level of sCD48 (8.60, IQR 6.40–10.45 ng/mL) was markedly higher than that in HC (4.90, IQR 4.12–6.67 ng/mL; $P<0.0001$, Fig. 3A). sCD48 did not correlate with inflammation grades ($r=0.077$, $P=0.414$) and could not differentiate varying histological inflammation grades in this cohort (Fig. 3B). However, correlation existed between sCD48 and fibrosis stages ($r=0.223$, $P=0.017$, Fig. 3C), and there was statistical difference for sCD48 levels between patients with S1 ($n=50$) vs S2 ($n=31$) ($P<0.01$). We then categorized patients into groups with no to mild fibrosis (S0-1, $n=63$) and significant fibrosis (S2-4, $n=51$), and the sCD48 level was elevated in patients with significant fibrosis ($P=0.004$, Fig. 3D). Other parameters higher in the significant fibrosis group included IgG, PT, INR, APRI, FIB-4, and liver stiffness measurement (LSM) values by TE, whereas PLT was significantly lower (Supporting Table S6). sCD48, INR, and FIB-4 were further demonstrated to be independent variables for significant fibrosis (Supporting Table S6).

Then, the predictive value of sCD48 for significant fibrosis was investigated (Fig. 3E; Supporting Table S7). sCD48 possessed a similar AUC (0.655) with those of LSM by TE (0.671), APRI (0.663), and FIB-4 (0.665). Scores combining LSM and sCD48 demonstrated an incremental increase in AUC (0.738). Nevertheless, the difference of AUC between the combined score and LSM was nearly but not significant ($P=0.0822$). In brief, for AIH patients at re-evaluation biopsy, sCD48 could predict significant fibrosis, and it might modify the diagnostic value of TE.

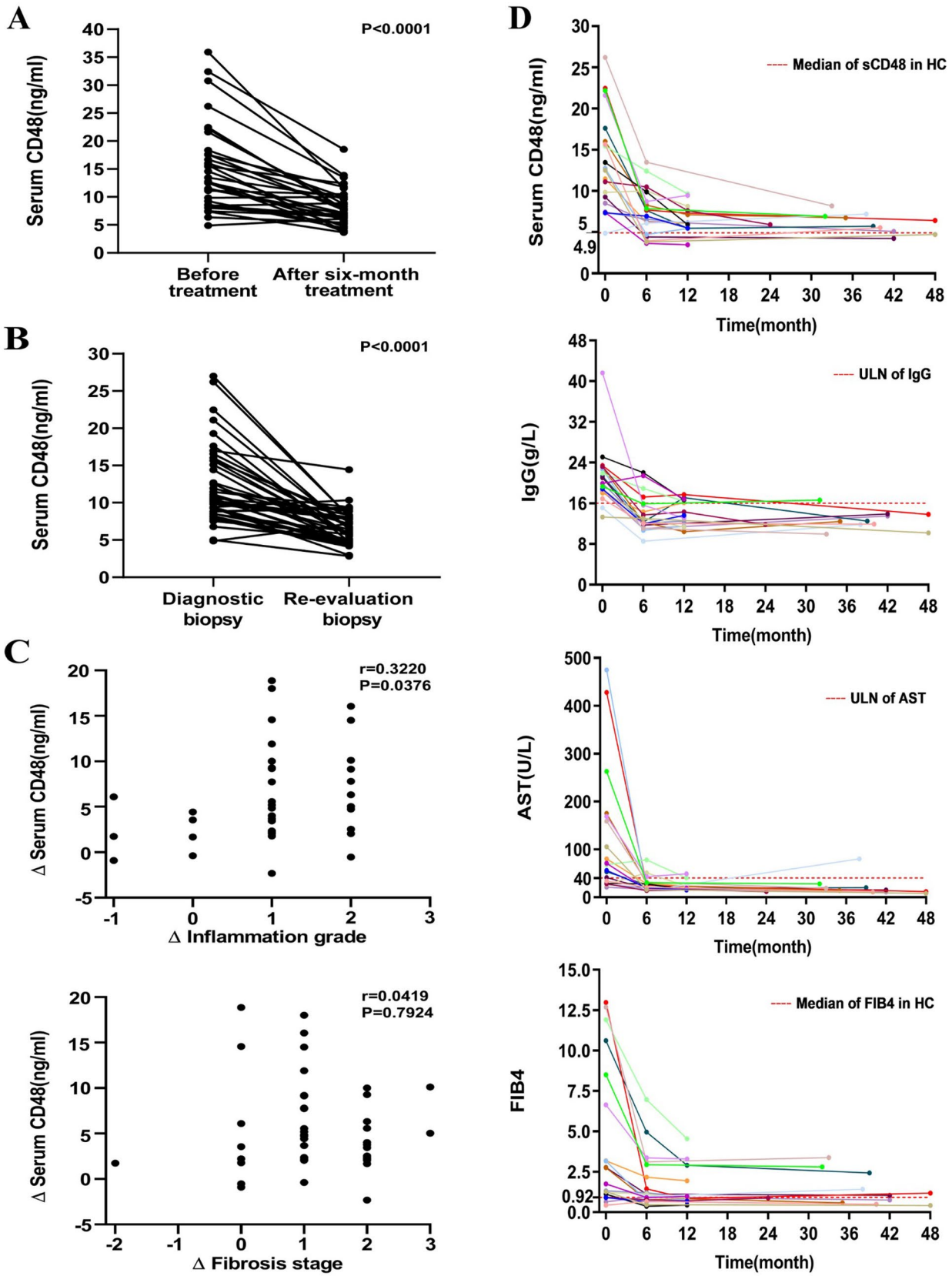
CD48 Expression was Higher in AIH Liver Tissues

Compared with HC, hepatic expression degree of CD48 was significantly upregulated in AIH ($P<0.0001$), NAFLD ($P=0.0036$), and PBC patients ($P=0.0004$). However, in contrast with both NAFLD and PBC patients, AIH patients manifested a higher CD48 expression (both of the $P<0.0001$, Fig. 4A). Moreover, hepatic CD48 expression degree showed strong associations with histological inflammation grades ($r=0.725$, $P<0.0001$) and fibrosis stages ($r=0.534$, $P=0.0007$, Fig. 4B). It also correlated with ALT and AST (Fig. 4C). Besides, in AIH patients with paired diagnostic and re-evaluation biopsy, degree of CD48 expression disclosed an evident decrease after treatment ($n=11$, Fig. 4D). Notably, degree of hepatic CD48 expression was related to sCD48 level ($r=0.616$, $P<0.0001$, Fig. 4E) and Δ serum CD48 and Δ hepatic CD48 expression degree between paired biopsies were also associated ($r=0.651$, $P=0.0345$), which suggested the increased sCD48 originated from the inflammatory microenvironment.

A Brief Review of Serum Markers in AIH

Noninvasive tests are of great importance for the diagnosis and management of AIH. Despite the fact that serum aminotransferases, IgG and autoantibodies are well-established diagnostic, predictive, and therapeutic markers, there are still unmet needs in feasible biomarkers for accurately monitoring treatment responses and individualizing therapies [2, 8]. Recent researches in pathogenetic mechanisms of AIH rendered the emergence of serum markers with potential efficacy in the clinical practices (Table 4), which were briefly outlined herein.

The dysregulation of the immune system is the main cause of AIH pathogenesis. Accordingly, among serum markers indicative of disease activities were several molecules involved in the immune regulation, such as sCD163 and sTIM-3 [30, 34]. It was reported that in acute AIH patients, the level of sCD163 was sixfold that in patients with complete response to standard treatment (9.50 vs 1.62 mg/L) and sCD163 positively correlated with ALT, IgG, and bilirubin [30]. The circulation level of some cytokines and their receptors could also reflect the disease activity, such as IL-33 and sST2 [41]. Liang et al. found that the IL-33 level was elevated in AIH patients relative to HC, and its level was associated with IgG [41]. Moreover, the levels of many markers of disease activities were decreased after immunosuppressive treatment (Table 4), but this observation should be interpreted with caution, as the decreased


A
P<0.0001
Serum CD48(ng/ml)
Before treatment
After six-month treatment
B
P<0.0001
Serum CD48(ng/ml)
Diagnostic biopsy
Re-evaluation biopsy
C
r=0.3220
P=0.0376
Δ Serum CD48(ng/ml)
Δ Inflammation grade
r=0.0419
P=0.7924
Δ Serum CD48(ng/ml)
Δ Fibrosis stage
D
Median of sCD48 in HC
Serum CD48(ng/ml)
Time(month)
ULN of IgG
IgG(g/L)
Time(month)
ULN of AST
AST(U/L)
Time(month)
Median of FIB4 in HC
FIB4
Time(month)

◂**Fig. 2** sCD48 levels decreased after immunosuppressive treatment. **A** sCD48 levels in AIH patients before and after 6-month immunosuppressive treatment ($n=35$). **B** sCD48 levels in AIH patients between the paired diagnostic biopsy and re-evaluation biopsy ($n=42$). **C** Correlation between Δ serum CD48 with Δ inflammation grade and Δ fibrosis stage; Δ = the value of diagnostic biopsy–the value of re-evaluation biopsy. **D** The longitudinal change of sCD48, IgG, AST, and FIB-4 before immunosuppressive treatment at diagnostic biopsy, after treatment at 6 months and 12 months and re-evaluation biopsy ($n=20$). Median of serum CD48 levels and FIB4 in healthy controls of our study were 4.9 ng/mL and 0.92 respectively; ULN values of IgG and AST were 16 g/L and 40 U/L respectively

level might be a direct result of the medication rather than reflect the decline of the disease activity.

The majority of AIH patients respond well to the standard immunosuppressive regime [29]. However, those non-responders should be detected early and treated with individualized therapies to improve the prognosis. Markers able to differentiate treatment responses included vitamin D, anti-nucleosomes antibody, sPD-1, and sCD163 [9, 30, 33, 36]. For example, circulating sPD-1 level was higher in incomplete responders to standard treatment than that in responders (0.17 vs 0.11 ng/mL, $P=0.01$) [33].

Moreover, as the routine indices could not reflect the histology accurately, the emerging markers might also assist in the prediction of histological changes. Hiroki et al. demonstrated that the serum level of WFA^+-M2BP was positively associated with fibrosis stages and inflammation grades [11]. For advanced liver fibrosis (F3-4), liver cirrhosis (F4), and severe liver inflammation activity (A3), WFA^+-M2BP manifested an AUC of 0.747, 0.853, and 0.739 respectively [11]. Additionally, different from most studies recruiting immunosuppression-naive AIH patients [11, 49, 50], a recent study included patients attaining biochemical remission under ongoing immunosuppressive therapy [51]. This study found that cytokeratin-18, but not ALT and immunoglobulins, was significantly higher in patients without histological remission (mHAI ≥ 4) than those with histological remission (mHAI ≤ 3), indicating that cytokeratin-18 might be helpful in selecting patients for re-evaluation biopsy when considering immunosuppression withdrawal [1, 51].

Despite the signs of progress in biomarker research, it was noteworthy that none of the markers listed (Table 4) has been suggested by the current guideline [2]. The low

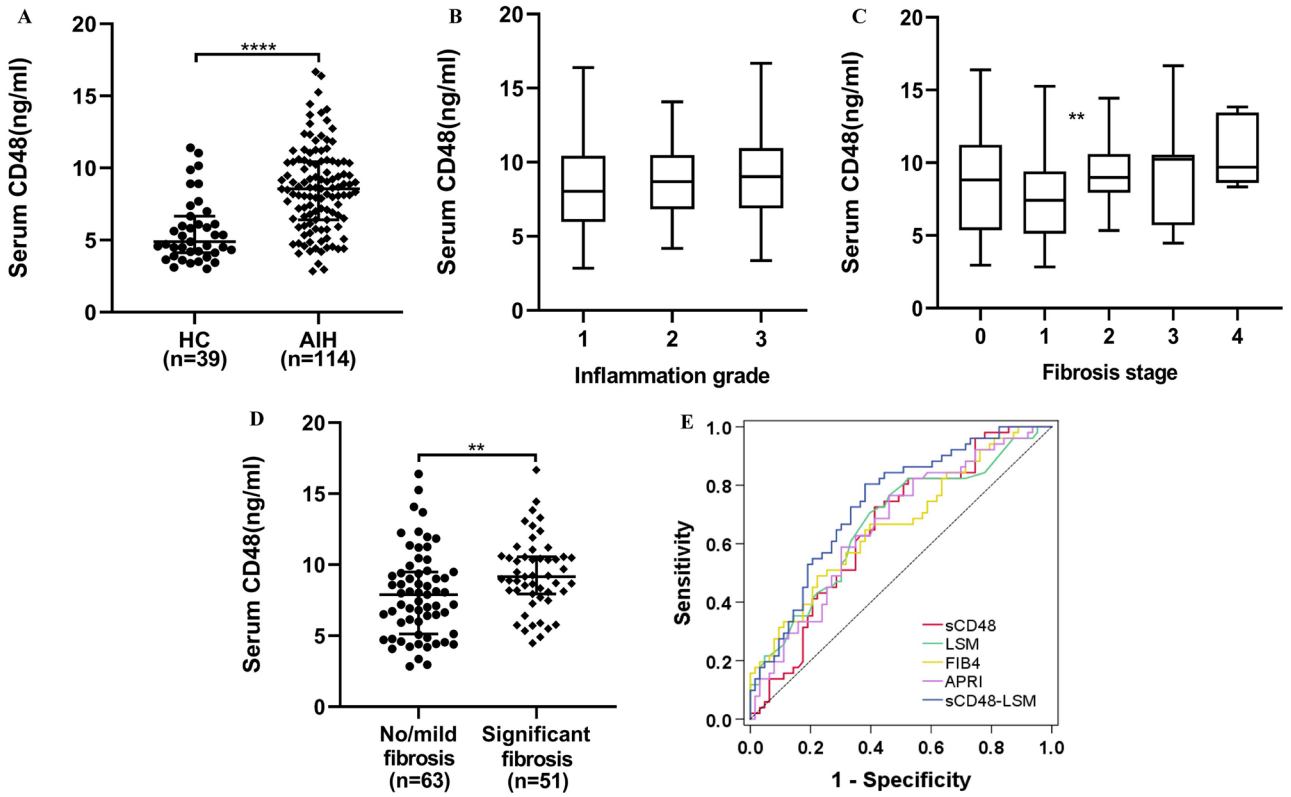


Fig. 3 sCD48 levels were elevated and could predict significant fibrosis in AIH patients at re-evaluation biopsy. **A** sCD48 levels in AIH patients at re-evaluation biopsy ($n=114$) and healthy controls ($n=39$). **B** Correlation between sCD48 levels and inflammation grades ($r=0.077$, $P=0.414$) in AIH patients at re-evaluation biopsy. **C** Correlation between sCD48 levels and fibrosis stages ($r=0.223$, $P=0.017$). $^{**}P<0.01$ for patients with S1 vs S2. **D** sCD48 in patients with no to mild fibrosis (S0-1) and significant fibrosis (S2-4). **E** ROC of sCD48, LSM, APRI, and FIB-4 for detecting significant fibrosis. Bars or boxes represented the median with interquartile range. $^{****}P<0.0001$, $^{**}P<0.01$

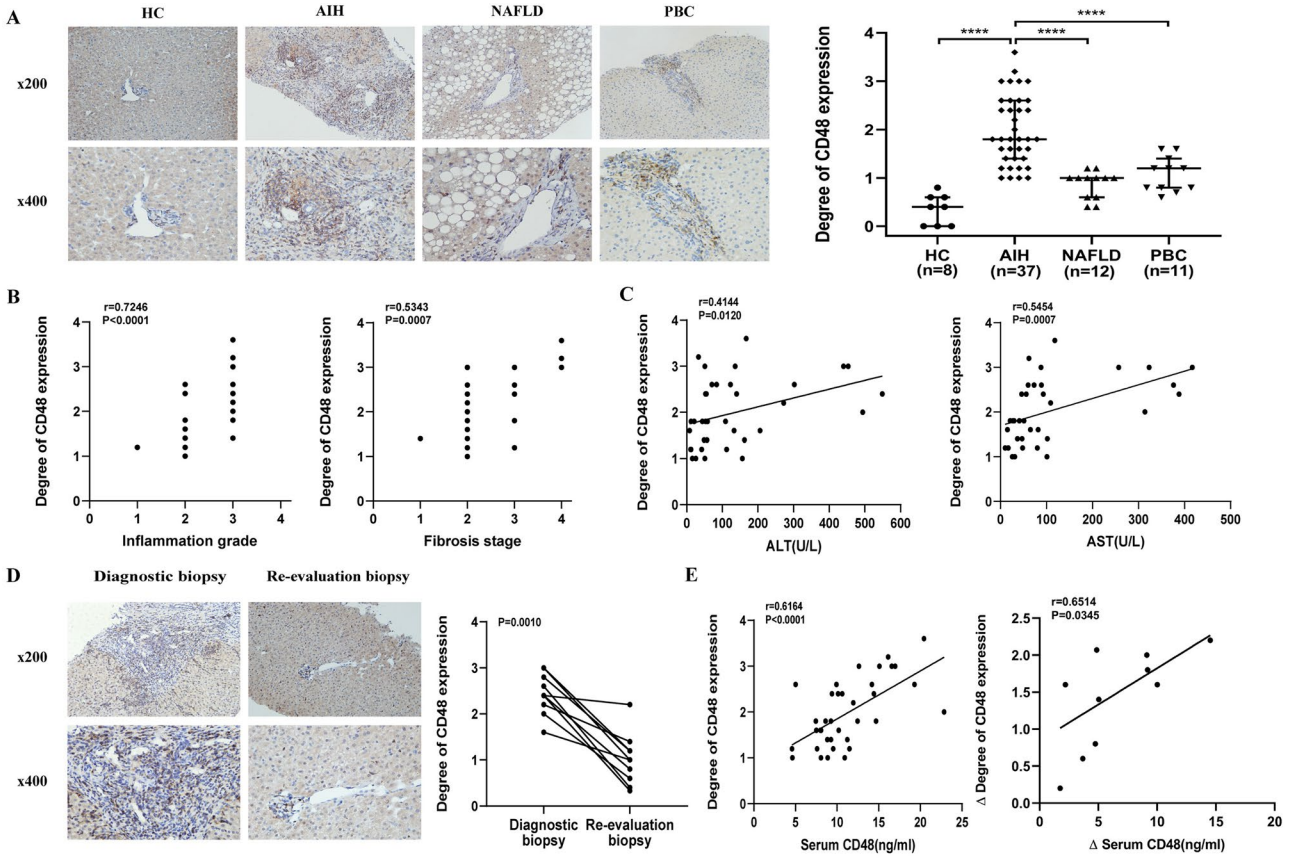


Fig. 4 Hepatic CD48 expression was upregulated in AIH patients and decreased after immunosuppressive treatment. **A** Hepatic CD48 expression in naïve AIH patients ($n=37$), NAFLD patients ($n=12$), PBC patients ($n=11$), and healthy controls ($n=8$). **B**, **C** Correlation between degree of hepatic CD48 expression with inflammation grades and fibrosis stages **B**, ALT and AST **C**. **D** Hepatic CD48 expression in AIH patients at paired diagnostic biopsy and re-evaluation biopsy ($n=11$). **E** Correlation between hepatic CD48 expression with sCD48 level ($n=37$), and correlation between Δ hepatic CD48 expression with Δ serum CD48 ($n=11$); Δ = the value of diagnostic biopsy–the value of re-evaluation biopsy. Bars represented the median with interquartile range. ****$P<0.0001$

rate of translation of these researches into clinical practice was mainly attributed to challenges and weaknesses in study design, especially the limited cases included and the lack of validation. Notwithstanding, we envision that with more rigorous study designs and the integration of different omics technologies [10, 45, 53], further research will promote the application of novel biomarkers into the clinical management of AIH patients.

Discussion

In this study, we reported that the sCD48 level was significantly elevated in immunosuppressive treatment-naïve AIH patients, similar to asthma and Sjögren's syndrome [18, 19]. Given the association between sCD48 levels and histological inflammation and fibrosis, we rigorously investigated the value of sCD48 and sCD48-based predictive models. For identifying significant inflammation (G3-4), sCD48-AIH-SI demonstrated an AUC of 0.813 in naïve AIH patients in the exploration cohort, superior to ALT, AST, and IgG. And sCD48 alone manifested an AUC of 0.748. A similar study by Gutkowski et al. developed an inflammatory score to predict high histological inflammation, which showed a perfect AUC of 0.93 [12]. But different from our study, they did not exclude the possible confounding effect of treatment. Because C-reactive protein included in their score was not available in majority of our participants, their score could not be evaluated in our cohort. Additionally, serum vitamin D and WFA^+-M2BP have also been reported to possess acceptable diagnostic ability for severe interface hepatitis and severe inflammation (A3, METAVIR system), with AUC of 0.744 and 0.739, respectively [9, 11].

For predicting advanced fibrosis (S3-4) in naïve AIH patients, sCD48 had a similar ability with APRI and FIB-4; the combined score sCD48-AIH-AF showed an improved AUC of 0.750 and 0.788 in the exploration and validation cohort respectively. APRI and FIB-4 have been tested for

Table 4 Putative serum biomarkers for disease progression and treatment responses in AIH patients

Biomarkers	Indicative values	Key points	Refs
sCD163	Disease activity, treatment responses	sCD163 levels were lower in AIH patients with complete response to standard therapy than both patients with an incomplete response and with acute disease; sCD163 levels decreased during prednisolone treatment	[30]
sPD-1	Disease activity, treatment responses	AIH patients with active disease and the incomplete responders to standard treatment possessed elevated sPD-1 levels compared with the responders and HC, but sPD-1 levels in responders were non significantly higher than HC	[33]
sTIM-3	Disease activity	sTIM-3 were elevated in AIH patients compared with CHC patients, PBC patients, and HC, and the level of sTIM-3 decreased after steroid treatment	[34]
Galectin-9	Disease activity	Galectin-9 levels were higher in AIH patients than CHC patients and HC; galectin-9 levels were associated with ALT and Tbil; galectin-9 levels were down-regulated by corticosteroid therapy	[35]
Anti-nucleosome	Diagnosis, disease activity, treatment responses	Anti-nucleosome levels were higher in AIH but not CHB and CHC patients relative to HC; the positive prevalence of anti-nucleosome was 71.1% in AIH; anti-nucleosome levels were significantly lower during remission than that during flares; the rate of relapse was significantly higher in patients with a slower reduction rate of anti-nucleosome	[36]
Anti-rib P	Prognosis	Moderate to high titers (> 40 U) of anti-rib P antibody were found in 9.7% of AIH patients and none of HC; positivity of anti-rib P might be a prognostic indicator for cirrhosis	[37]
IL-2	Treatment responses	Higher baseline IL-2 levels might predict biochemical remission in pediatric AIH patients	[38]
sIL-2R	Disease activity	sIL-2R levels were higher in AIH patients with highly active disease than those with mild activity; the sIL-2R level was correlated with ASGPR titer, and its level was decreased after immunosuppressive therapy	[39]
IL-6, IL-8	Disease activity	IL-6 and IL-8 levels were higher in AIH patients than in HC; their levels decreased after achieving remission in AIH patients; higher IL-8 levels were associated with HLA*DRB15	[40]
IL-33, sST2	Disease activity	IL-33 and sST2 levels were higher in AIH patients than HC. After treatment, IL-33 and sST2 levels decreased; IL-33 positively correlated with ALP, GGT, and IgG	[41]
TNF-α, adipokines	Disease activity, histological changes	Adiponectin, lectin, and TNF-α levels were higher in AIH patients than HC; TNF-α levels were associated with ALT and decreased after treatment; adiponectin levels increased with Child score and advancing fibrosis stage	[40–42]
Vitamin D	Disease activity, prognosis, treatment response, histological changes	25(OH)D levels were lower in AIH than HC; 25(OH)D levels decreased in parallel with the increase in grades of interface hepatitis and fibrosis stages; lower 25(OH)D levels were associated with poorer treatment response; severe vitamin D deficiency at presentation was associated with developing cirrhosis, liver transplantation, and liver-related deaths	[9, 43]
WFA^+-M2BP	Disease activity, histological changes	WFA^+-M2BP levels were elevated in AIH compared with SLE and CHC patients, and their levels were decreased after steroid treatment; WFA^+-M2BP levels increased in parallel with the increase in liver fibrosis stages and inflammation activity; it manifested a satisfying predictive value for advanced fibrosis(F3-4), cirrhosis(F4), and severe inflammation activity(A3)	[11, 44]
SAA1	Histological changes	Relative to HC, SAA1 levels in AIH were higher, but not in CHB and CHC patients; SAA1 level was related to different grades of liver inflammation	[45]

Table 4 (continued)

Biomarkers	Indicative values	Key points	Refs
sICAM-1, IP-10	Disease activity	sICAM-1 and IP-10 levels were elevated in AIH compared with SLE and CHC patients, and their levels were decreased after steroid treatment	[44]
miR-21, miR-122	Disease activity, histological changes	MiR-21 and miR-122 were upregulated in AIH in relation to CHC patients and HC, and their levels were decreased after glucocorticoid treatment in AIH; AIH patients with cirrhosis and advanced fibrosis possessed lower miR-21 and miR-122 levels; miR-21 levels positively correlated with liver necroinflammation grades	[46]
GP73	Disease activity, histological changes	GP73 levels elevated in parallel with the increase in liver necro-inflammatory grades and fibrosis stages; GP73 levels correlated with inflammatory activity grades and was an independent predictor of liver necroinflammation	[47, 48]
ACE	Histological changes	ACE levels were higher in AIH patients than HC; ACE levels increased in parallel with the increase in hepatic fibrosis stages and had a good predictive performance for different fibrosis stages	[49]
ADA	Histological changes	ADA levels were higher in AIH patients than HC; ADA levels were associated with interface hepatitis grades; ADA levels had a good diagnostic performance for severe interface hepatitis	[50]
CK-18	Histological changes	In AIH patients with biochemical remission and under immunosuppressive treatment, higher CK-18 level could distinguish patients with persistent histological activity from those achieving histological remission (mHAI ≤ 3)	[51]
EN-RAGE, sRAGE, EN-RAGE/sRAGE	Disease activity, histological changes	EN-RAGE and EN-RAGE/sRAGE levels were higher in AIH patients than HC, and higher in AIH patients with cirrhosis than those without, while sRAGE levels were lower in AIH patients than HC and lower in those with cirrhosis; EN-RAGE and EN-RAGE/sRAGE decreased while sRAGE increased after treatment	[52]

ACE angiotensin-converting enzyme, *ADA* adenosine deaminase, *Anti-nucleosome* autoantibodies against nucleosomes, *ASGPR* anti-asialoglycoprotein receptor *Anti-rib P* autoantibodies to ribosomal P proteins, *CK-18* cytokeratin-18, *GP73* Golgi protein 73, *EN-RAGE* extracellular newly identified receptor for advanced glycation end products binding protein, *Gal-9* Galectin-9, *SAA1* serum amyloid A1, *sPD-1* soluble programmed death-1, *IP-10* interferon-γ-inducible protein 10, *sRAGE* soluble receptor for advanced glycation end products binding protein, *sICAM-1* soluble intercellular adhesion molecule-1, *WFA^+-M2BP* Wisteria floribunda agglutinin positive Mac-2-binding protein, *25(OH)D* 25-hydroxyvitamin D

diagnosing fibrosis in different AIH cohorts [11, 23, 28]. Consistently, our study also reported a modest predictive ability of APRI and FIB-4 for distinguishing advanced fibrosis. TE shows reliable performance for diagnosing advanced fibrosis in AIH patients. A well-designed study by Hartl et al. identified at least 6-month treatment as the requirement for eliminating the disturbance caused by hepatic inflammation [7]. However, the comparison of sCD48 with TE in naïve patients could not be performed because TE was unavailable in 99 and 25 cases in our exploration and validation cohort, respectively.

Prednisone/prednisolone alone or in combination with AZA remains the first-line treatment and works well for most AIH patients [2]. Biochemical remission was attained in most patients within 12 months after immunosuppression [29]. In line with this, our study showed that AST and IgG decreased sharply after treatment and normalized in most patients at 12 months after treatment and re-evaluation biopsy. Moreover, Δ serum CD48 positively correlated with Δ inflammation grade between paired diagnostic and re-evaluation biopsies, which indicated the decrease of sCD48 might reflect the resolution of histological inflammation during treatment. In the study of sCD163, a similar longitudinal change of sCD163 has been observed, but the level of sCD163 has been followed for only 6 months [30]. sCD48 might not indicate biochemical remission for AIH patients, but as mentioned above, vitamin D, and sPD-1 could (Table 4). In addition, TE was used to monitor fibrosis development during treatment [31]. Data of TE during follow-up were not available in this study, but the decrease of sCD48 paralleled with that of FIB-4. Thus, the regular detection of sCD48 in AIH patients might help in monitoring disease activity during treatment.

Due to the harms of long-term immunosuppression, treatment withdrawal should be considered for AIH patients with sustained biochemical remission for at least 2 years

[2]. Though pre-withdrawal liver biopsy is not mandatory, histological findings, both inflammation activity and fibrosis, are important references for withdrawal [1]. In our re-evaluation biopsy cohort, sCD48 in AIH patients maintained a higher level than HC. Moreover, sCD48 was an independent predictor for significant fibrosis and it might modify the diagnostic performance of TE, which is emerging as a helpful indicator in the withdrawal decision [2, 31]. Therefore, sCD48 possibly aided in the treatment withdrawal.

Previous studies reported CD48 was involved in the regulation of $CD8^+$ T cells and NK cells for chronic hepatitis B and hepatocellular carcinoma respectively [14, 15]. In our study, hepatic CD48 expression in AIH was upregulated on immune cells surrounding the portal tract; its expression manifested significant associations with histological inflammation and fibrosis. Moreover, CD48 decreased after immunosuppressive treatment. Such observations suggested the possibility of CD48 as a novel therapeutic target for AIH, as reported in multiple sclerosis [32]. We have demonstrated that $CD8^+$ tissue-resident memory T (TRM) cells were possibly significant contributors to the pathogenesis and persistence of AIH [27]. Additionally, our preliminary results (not published) found that CD244, the receptor for CD48, was upregulated in $CD8^+$ TRM cells. Thus, we postulated that the enriched hepatic CD48 expression on immune cells (especially on antigen presentation cells) might exert a regulatory role in the pathogenic function of $CD8^+$ TRM cells via CD244, like the reported regulation of NK cells by monocytes in hepatocellular carcinoma [14]. This ongoing work in our laboratory might provide further mechanistic evidence for the possible utility of CD48 as a novel therapeutic target for AIH.

Noteworthy, the association between hepatic CD48 expression and sCD48 level, and the association between Δ serum CD48 level and Δ degree of hepatic CD48 expression of the paired biopsies indicated that sCD48 potentially originated from the inflammatory liver in AIH, consistent with the theory that soluble CD48 was generated from the cleavage of membrane CD48 [16]. It should be noted that the role of either CD48 or sCD48 has also been reported in other immune-related diseases [13, 17–19]. Thus, despite the observation that sCD48 levels in AIH were significantly higher than those in PBC and NAFLD patients, and concurrent autoimmune/allergic diseases had no significant impact on sCD48 levels in AIH (Supporting Fig. S1C), our results could not reach the conclusion that sCD48 was of specific value for AIH. Though the reported concentration of sCD48 detected in asthma (962–3835 pg/mL, $n = 281$) [18] and Sjogren's syndrome (about 750–5250 pg/mL, $n = 58$) [19] were seemingly lower than AIH patients in our cohorts (2.19–41.10 ng/mL for naïve patients ($n = 221$) and 2.83–16.67 ng/mL for patients as re-evaluation biopsy ($n = 114$)), the direct comparison was obviously affected by the different detection procedures and sample quality as well as limited subjects. Therefore, the specificity and value of sCD48 for AIH needed to be further investigated in multicenter cohorts of large scale, including subjects of other immune-related diseases without AIH.

In summary, with cohorts of AIH patients at different treatment stages, we demonstrated the possible value of sCD48 and sCD48 based models for pretreatment and after treatment histological assessment, as well as disease activity monitoring during treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12016-022-08935-z.

Acknowledgements We are grateful for all the participants enrolled in the study.

Author Contribution X. M., Q. W., and Q. M. designed the study. M. H., Z. Y., and Y. L. performed the experiments. B. H., N. C., R. W., Y. W., B. L., Y. L., J. L., H. W., and Q. Q. collected samples. M. L., J. Z., R. C., Z. L., Q. L., Y. C., X. X., M. L., and R. T. analyzed the data. M. H. and Q. W. wrote the manuscript. X. M. reviewed the manuscript.

Funding This work was supported by the National Natural Science Foundation of China grants (#82130017, 81830016 to XM, #82070581, 81770564 and 81790634 to QW, #81770563 to QM, #81800504 to ML, #81922010 and 81873561 to RT), Shanghai Sailing Program (No. 18YF1412900 to ML).

Declarations

Conflict of Interest The authors declare no competing interests.

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